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J.B. BATEMAN

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This report provides a breadkown of topics considered at a symposium on "Electrical Phenomena at the Level of Biological Membranes" constituting the 29th International Meeting of the French "Société de Chimie Physique". The interdisciplinary nature of the symposium is illustrated by summarizing the proceedings in terms of "biological" and "non-viable" systems. The attempt is made to present the substance of the various contributions against a background that will make the report accessible to the general reader. It is suggested that the admirable goal of a truly interdisciplinary exchange was not

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achieved despite the evident efforts of some of the speakers to escape from the limitations of their own specialities.

ELECTRICAL PHENOMENA IN BIOLOGICAL MEMBRANES: A SYMPOSIUM

by

J.B. BATEMAN

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1. INTRODUCTION

The twenty-ninth international gathering of the Société de Chimie Physique (Université de Paris-Sud, Orsay, 12-15 October 1976) devoted itself to the question of electrical phenomena at the level of organization of biological membranes. Some impressions have been recorded in European Scientific Notes (*ESN* 31-5:172). This report supplements that short article with a more objective indication of the technical coverage.

The full title of the symposium was: "Phenomènes Electriques au Niveau des Membranes Biologiques", suggesting rather more than the abbreviated title in this summary. It suggests, indeed, that non-biological membranes of a quasi-biological quality could be discussed side-by-side with actual living membranes. So the main objective, although unstated in so many words, could have been supposed to be an exchange of information among biologists, physical chemists and physicists within the general framework of electrical phenomena in membranes. It must have been hoped that the impenetrable barriers of specialist jargon, not to mention different languages and national usages, would in fact be penetrated by closeting everybody in the same room and asking them to address each other by means of formal reports, informal discussion, and perhaps while dining together afterwards. Although in my opinion the desired degree of technical compatibility was not achieved, the program planners did succeed in getting a fair amount of cross-talk. Perhaps this was the most that could be expected short of elaborate preliminary briefings to which many of the participants would probably have refused to submit themselves.

In this report, in the interest of clarity coupled with self-indulgence, I have tried to unscramble the mixture, dividing living from non-living systems and, within each category, subdividing according to the nature of the biological phenomenon under consideration or the nature of the non-living systems that are presumed (having been included in the program) to offer something of biological relevance. The result will be seen in the table of contents on page i. There, the numbers in brackets show the number of papers devoted to the several areas.

2. BIOLOGICAL SYSTEMS

General Properties of Biological Membranes

E.L. Benedetti (U. Paris VI) gave an admirable paper on the general properties of biological membranes. He presented the plasma membrane as a receiving-transmitting system consisting of an asymmetric lipid

bilayer upon which specific properties are conferred by the non-covalent, often asymmetric, incorporation of appropriate amphipathic glycoproteins or oligomeric peptides. The necessary ordering of these dispersed substances within the fluid continuous phase is brought about and stabilized in some way, perhaps by chemical cross-linking or by anchoring to a contractile protein similar to actin, a substance intimately involved in muscular contraction. On the other hand, some important cellular processes, for example those involved in immune reactions, are accompanied by lateral migration so that there must be some sort of balance, varying with the type of cell, between membrane fluidity and gelation. Benedetti chose to emphasize properties depending upon local or general mechanical stability, and several illustrations showed the dependence of membrane properties upon the protein component. There was some discussion of the possible role of actin, usually present in plasma membranes, in controlling changes of permeability and mediating the action of mitotic stimulants. Another important property of some cell membranes is associated with the presence of modular peptide subunits, a few of which may suffice to permit intercellular electric coupling. An initial membrane resistance of 10^4 - 10^5 ohm cm^{-2} may drop when cells come into contact to 5 - 9 ohm cm^{-2}, permitting transfer of information residing in substances of molecular weight as high as 1000.

In the case of the acetylcholine (ACh) receptor at the nerve-muscle junction, permeation is controlled by a pentameric or hexameric protein with a central pit; a change of conformation caused by binding of ACh transforms the pit into a channel capable of permitting passage of inorganic ions through the membrane.

Red Blood Cells

One of the two papers (A. Caprani, C. Deslouis and B. Tribollet: U. Pierre et Marie Curie, Paris) consisted of a discussion of the conditions under which polarographic measurements on a system containing red cells and fast redox diffusion tracers can yield information about ion transport through the cell membrane. In the other paper, J. Garnier and S.J. Singer (U. Paris-Sud, Orsay and U. Calif., La Jolla) drew attention to an interesting correlation between the effects of certain drugs on the chemical stimulation of adenylate cyclase activity in nucleated red cells, and the gross effect of these drugs on the cell membrane. Drugs of one class, of which dinitrophenol is an example, produce a crenate surface and are called crenators; those of the other class, which include tranquillizers and other cationic or amphipathic substances, cause invagination of the surface and are called cup-formers. These two physical effects are attributed to

differences in solubility in the asymmetric lipids of the cell membrane: the crenators dissolve in the outer lipid and cause the outer half of the bilayer to increase in area, while the cup formers dissolve in the inner layer. The unmatched expansions cause an outward convexity in the one case and concavity in the other. Crenators and cup formers both inhibit the stimulation of adenylate cyclase by catecholamines (isoproterenol was used) but they have opposite effects on the stimulation of the enzyme by fluorides; crenators inhibit while cup formers intensify it. Evidently the fluoride sites are differently situated on the inside and outside of the membrane so that the effects of the two classes of drug should cancel in this case, but should reinforce each other in the case of catecholamine stimulation. It was not made quite clear whether these predictions had been experimentally verified.

Oscillatory Phenomena in Plant Cells

Oscillatory bioelectric potentials in the giant plant cell *Nitella* were described by Z. Damjanović in a paper by C. Radenović, Z. Vucinić and the speaker (U. Belgrade, Yugoslavia). The oscillations induced by alkali cations at concentration 10 mM showed characteristic differences of frequency, damping, interval between bursts and overall duration. Potassium, for instance, produces only a few spikes of very high frequency; cesium, a damped oscillation with constant intervals; lithium causes oscillations only after a latency which may last as long as 30 minutes. Very little evidence was offered as to mechanisms and possible concomitant impedance variations. Discussion brought out some resemblances to the behavior of mineral electrochemical models studied particularly by Monnier.

Chemotaxis

Chemotaxis in the slime mold *Physarum polycephalum* is triggered, according to Y. Kobatake (Hokkaido U., Sapporo, Japan), by cell membrane depolarization. Kobatake was able to prepare spherical forms, diameter 50-100μm, by shaking the large protoplasmic aggregates or plasmodia in which the organism ordinarily exists. These spherical forms are amenable to measurement of membrane potential, zeta potential and chemotaxic response. It is found that for a large number of substances tested the concentration thresholds for depolarization and for chemotaxis (whether attractive or repulsive) are identical. The investigation continued along classical colloid-chemical lines, with establishment of decreasing thresholds for univalent cations in the slightly unusual series H-Li-K-Na-Rb-Cs-NH_4, and verification of a lyotropic anion series for each cation. The thresholds were usually modified in a rather erratic manner by sugars, being raised by the aldohexoses glucose

and mannose and by the disaccharides maltose and sucrose, lowered by the pentose ribose, and unaltered by the aldohexose galactose. Alone among the univalent cations, NH_4^+ had the same threshold for all of these sugars. The normal alcohols were increasingly effective with increasing chain length. Kobatake attempted to relate all these effects to changes in the structure of the water surrounding the various reactants, but it seemed that other factors, such as solute adsorption and differences in utilization of the various sugars, had not been thoroughly studied. No doubt many of the apparently specific effects of organic molecules of similar chemical constitution are due to accidental dimensional matching.

Nerve

All of the papers on nerve were about sodium and potassium channels; one was theoretical, one dealt with the squid giant axon and--in common with the remaining three--with medullated nerve, which is electrically accessible only at certain nodal points along its length, the nodes of Ranvier. The contents of the theoretical paper can be judged only by the abstract, in absence of the author, Yu. A. Chizmadzhev (USSR Academy of Sciences). The abstract promises a phenomenological model of single file ion transfer through open channels and calculations of the main thermodynamic and kinetic parameters and energy profiles. A scheme of channel conformation control by sets of interacting dipoles is suggested as a basis for calculation of "the basic equilibrium and kinetic characteristics of membranes--gating currents, dependence of characteristic times on the potential, electric fluctuation spectrum, and ionic current".

The experimental papers on nerve seemed to be addressed mainly to fellow axonologists, and the untutored listener may have wondered why nothing was said about the main function of nerve, namely to propagate electrochemical information rather in the manner of an electric cable. E. Rojas (Ecole Normale Supérieure, Paris) made a valiant introductory effort at establishing a perspective but soon became carried away by the technicalities of his own work. It might have been well if he had more clearly stated that the essential electrical preliminaries to the triggering of an impulse occur even with sub-threshold stimuli and can thus be studied--indeed, must be studied--exhaustively before the propagated disturbance itself can be fully understood at the molecular level. In what follows I draw upon a nodding acquaintance with the prior work of Rojas in the laboratory of R.D. Keynes at Cambridge, in order to bolster a very imperfect understanding of his more recent conclusions as presented at Orsay.

Resting nerve contains a lower concentration of sodium ions and a higher concentration of potassium ions than the surrounding medium. Once established by performance of metabolically derived work, these differences are maintained, more or less, by a membrane structure which prevents passage of sodium and chloride ions while being permeable to potassium. The result is a quasi-equilibrium Nernst concentration-cell potential difference between inside and outside of the nerve, the inside being negative to the outside by some 65 mV. The primary event in production of an electrical transient upon application of a depolarizing voltage pulse is the opening of a molecular "gate" guarding the sodium ion channels. Sodium ions can then flow passively down the concentration gradient. The sodium current is, however, transient, being limited by intervention of a process known as inactivation. The sodium current thus decreases to zero, but is followed by leakage of potassium ions from the inside of the nerve, through a different set of channels. Rojas' work has dealt mainly with the initiation and inactivation of the sodium current. The initial gating process is essentially that of charging or discharging a condenser the dielectric of which is capable of change of dipolar configuration between two or more electrostatic energy levels. Fortunately this can be studied in isolation since the sodium ion channels can be blocked by tetrodotoxin (TTX), a cyclic polypeptide. It is found that the time constants of the displacement current, measured in presence of TTX, follow the same course of variation with variation of applied depolarizing voltage as those of the sodium current (in absence of TTX, of course) and are of roughly the same magnitude. This seems to identify the condenser charging process with the opening of the sodium ion gate. Use of another procedure makes it possible to prevent the sodium ion current inactivation. This is done by perfusing the squid giant axon intracellularly with a proteolytic enzyme complex, pronase, the activity being associated with a highly substrate-specific constituent, alkaline proteinase b. The new result that emerged from Rojas' talk at Orsay seems to be that sodium current inactivation is not brought about by a corresponding reversal of the gating mechanism as manifested by displacement current kinetics. Presumably a secondary mechanism operating at some other point within or around the sodium channel is responsible.

In two of the remaining three papers on the node of Ranvier certain aspects were reviewed. I missed B. Neumcke's (U. Saarland, Homburg/Saar, FRG) paper and can only report, from the abstract, that it dealt with the specific and unspecific (sic) negative surface charges for sodium and potassium ion transport which occupy sites on the outer surface of the node. The specific sites offer a single electronic charge per channel. They probably serve to initiate passage of Na^+ and K^+ ions by transient binding and are neutralized by protons or other cations. The "unspecific" negative charges at a density of about

one per (20 Å)^2 (sic) contribute a negative component of 48 mV to the external surface potential and, being modified by ions in the external solution, exercise some control on the electric field strength in the membrane which in turn affects the voltage-dependent gates to the ion-passing channels. W. Ulbricht (U. Kiel, FRG) reviewed the effects of selective channel-blocking agents, setting out from Bertil Hille's conception of the mouth of the sodium channel as a negatively charged selectivity filter lined with oxygen atoms which form hydrogen bonds with specific blocking agents such as tetrodotoxin (TTX) and saxitoxin (STX). Local anesthetics such as procaine, on the other hand, act at the inner end of the channel. The stoichiometry and the kinetics of the reactions are consistent with the conclusion that TTX and procaine act independently, the channel being blocked if either or both receptors are occupied. The potassium channels are selectively and reversibly blocked by the tetraethylammonium (TEA) ion applied externally. Selective blockage is produced also by 4-aminopyridine (4AP), but the binding site appears to be within the channel and to be inaccessible if the gate is closed.

In a much more detailed paper, C. Bergman and J.M. Dubois (Ecole Normale Supérieure, Paris) gave experimental evidence in favor of the hypothesis that the potassium ion gate is opened by combination of potassium ions in the external medium with a negatively charged site at the external mouth of the channel. One postulates a stoichiometric reaction

$$S + K \rightleftharpoons SK$$

where S denotes the closed channel receptor, K is potassium ion, and SK is the open channel receptor complex. An apparent dissociation constant can be written:

$$G = [S][K]/[SK]$$

where [S] is concentration of closed channels, [SK] the concentration of open channels and [K] is the potassium ion concentration in the medium in which the nerve is immersed. Putting

$$[S] = [S]_o - [SK]$$
$$g = k[SK]$$
$$g_{max} = k[S]_o$$

leads to

$$1/g = G/g_{max}\,[K] + 1/g_{max}$$

Here g by definition is proportional to the fraction of open channels and hence to the potassium conductance of the membrane under given conditions, while g_{max} is the conductance when all channels are open. The equation, resembling the Langmuir (or Michaelis - as the authors prefer) adsorption isotherm, would be open to experimental test if g could be measured, and the apparent dissociation constant determined. How can g be measured? For a given external potassium concentration the equilibrium membrane potential E_o is given by the Nernst equation (if one ignores criticisms of this use of the equation) from the ratio of the potassium concentrations inside and outside the nerve. If the membrane potential is maintained at some more negative value E, using the well-known "voltage clamp" feedback technique, there will be an inward flow of potassium ions under a driving force $E - E_o$ giving rise to a current i. Thus

$$g = i/(E - E_o)$$

Using this method, Bergman and Dubois obtain a series of straight lines plotting $1/g$ against $1/[K]$ for various constant values of E, confirming the adsorption hypothesis for channel opening and confirming that increased external potassium acts qualitatively in the same sense as membrane depolarization. Since the potassium effect is embodied in the apparent dissociation constant G, the interdependence of external potassium and membrane potential can be expressed by plotting G against E. This takes the form of two intersecting exponentials, suggesting either the involvement of two receptor sites per channel or two species of site. Given the foregoing evidence for potassium binding, an extension of the model adjusted empirically makes it possible to calculate the voltage dependence of the potassium current as a function of external potassium concentration over a range of membrane depolarization potentials on the positive side of the relevant Nernst equilibrium, so that potassium flow is in the reversed direction--that is, it leaks out of the nerve, as it does in the second phase of the ordinary action current of stimulated nerve. In the modified model the closed gate is supposed to exist in two states, charged and uncharged (inactivated and activated) and the reaction scheme becomes

$$\underset{\text{closed}}{S \rightleftharpoons S^-} + K^+ \underset{\text{open}}{\rightleftharpoons SK}$$

The empirical equation based on this model gives a satisfactory representation of membrane current as a function of negative and positive values of E and of high and low external potassium concentrations, including the apparent negative resistance characteristic. A further piece of evidence in favor of adsorption at the outside end of the channel is that the effect of external potassium can be copied by cesium which is unable to pass through the membrane.

Postsynaptic Membrane

Transmission of excitation from motor nerve terminals to muscle is performed by acetylcholine (ACh). The nerve impulse causes this substance to be released from "quantal" packets or vesicles in the vicinity of the postsynaptic membrane. The substance is at once taken up by the membrane, where in sufficient quantity it causes the depolarization that acts as stimulus for muscular contraction or--in the case of the electric organs of the ray *Torpedo* and the eel *Electrophorus* -- production of an electric impulse. The ACh receptor is a protein which has been studied *in situ* and in purified form. Much recent progress in this direction has followed upon the success of J.P. Changeux (Inst. Pasteur, Paris) and co-workers in isolating the protein in milligram amounts from *Electrophorus*; and since Changeux gives a very persuasive lecture, it is not surprising that invitations are received to do so on many different occasions. At Orsay, as at Copenhagen the previous summer, he gave some background information on the ACh vesicles, proceeding then to describe the properties of the receptor in isolation and in membrane fragments, and winding up, as one should, with some summary conclusions.

The successful isolation of the receptor protein owes much to the fact that it combines specifically with the snake venom constituent α-bungarotoxin which can be used as a radioactive tracer during the purification. The receptors are solubilized by non-ionic and anionic detergents and purified by affinity chromatography. The yield is about 50 mg per kg electric organ. The product is a lipoprotein similar in its moderately hydrophobic character to rhodopsin, with the following properties:

molecular weight average	3.10^5 daltons
subunits	4.10^4 daltons and others
sedimentation constant	9.5 svedbergs
molecular radius	8.0 nm
Stokes radius	7 nm
isoelectric point:	
Electrophorus	5.3
Torpedo	4.6 - 4.8
shape	sphere or rod with central hydrophilic hole.

The functional properties are studied using physical techniques to prepare fragments of the postsynaptic membrane. By tagging with α-bungarotoxin and sedimenting in a sucrose gradient it is found that receptor protein accounts for 20-30% of total membrane protein. The receptor-rich fractions are covered with receptor protein packed closely at about 5.10^4 sites per sq. micron, sometimes in a sufficiently regular

array to give one or more equatorial X-ray reflections. The center-to-center distance is 8-9 nm and the thickness about 8 nm. These membrane fragments can form "bags" which have been used for measurement of membrane conductance and permeability. The rate of loss of ^{22}Na gives a measure of permeability under various conditions.

The summary conclusions were expressed in terms of a model showing the receptor in three interchangeable states: combined with antagonist, gate closed; combined with agonist, gate open; and desensitized, gate closed. The so-called "desensitized" state is reached either by displacing the antagonist with agonist and calcium ions or by the action of local anesthetic on the agonist-receptor complex. The postulated conformation change correlated with the gating process has been demonstrated by fluorescence spectroscopy.

This last point was taken up in some detail by H. Grűnhagen (Max Planck-Inst.f. Biophysikalische Chemie, Göttingen, FRG and Inst. Pasteur, Paris) in collaboration with Changeux and Iwatsudo. If I understand it, the basis of this work is provided by the fact that the fluorescent acridine dye quinacrine combines with the ACh receptor in the vicinity of the channel. The intensity of fluorescence is sensitive to changes in the environment such as conformational alterations in the receptor attendent upon its interaction with agonists or antagonists. Competitive antagonists such as curare cause a slow increase of fluorescence approaching a limiting value. Agonists on the other hand (e.g., ACh, nicotine or carbamyl choline) cause a large transient increase of fluorescence ("overshoot") which then settles down to the same limiting value as that found in presence of antagonist. The rapid component is eliminated by α-bungarotoxin and is so thought to be correlated with receptor activation and channel opening. The kinetics of the fast process have been followed by the stopped-flow method in which a solution containing membrane bound receptors and quinacrine is rapidly mixed with a mixture of effector (agonist) and quinacrine: incident radiation 295 nm, fluorescence monitored at 540 nm. The observed kinetics are incompatible with the simple model

$$R + E \rightleftharpoons RE$$

(R = receptor, E = effector), and the change of fluorescence is thought to be attributable to a subsequent change in the complex RE:

$$R + E \underset{k_2}{\overset{k_1}{\rightleftharpoons}} RE \underset{k_4}{\overset{k_3}{\rightleftharpoons}} R'E$$

From this, with some assumptions, Grűnhagen calculates the velocity constants k_3 and k_4, the two equilibrium constants and the activation

energies for binding and for isomerization. The reaction appears to be too slow to correspond to channel gating which takes only a few milliseconds and, therefore, does not offer a final interpretation of the molecular foundation of the process. The *in vitro* kinetics may differ from those *in vivo* and may perhaps be altered by quinacrine.

The third paper on electrical phenomena at a post-synaptic membrane provided some insight into the difficulties of research on systems less readily accessible than the classical cholinergic end-plate. B.L. Ginsborg (U. Medical School, Edinburgh, Scotland) introduced the subject by reviewing briefly the problems met with when one tries to interpret membrane conductance changes in terms of a switched (gated) combination of EMF and resistance inserted in parallel with the resting resistance and membrane potential. Questions arise as to the multiplicity of channels serving to transmit different ions and the possible effects of agonists in causing channels to close rather than to open. Technical advances such as those offered by analysis of changes of electrical membrane noise brought about by transmitter molecules and drugs are limited to short term experiments and, therefore, to study of rapidly acting agents. Special difficulties arose in Ginsborg's own work on a kind of secretory activity in which dopamine is involved. The cells of the salivary gland of the cockroach, *Nauphoeta cinerea*, are too small to admit more than one microelectrode so that electrodes have to be inserted in adjoining cells, and the electrical phenomena observed have to be interpreted on the assumption that the cells within the acinus are essentially in electrical contact with each other while the resistance between the cytoplasm and the external solution in the duct leading to the acinus is relatively large. The effects of nerve stimulation, of perfusion with 10^{-6}M dopamine and of change of external potassium concentration were studied by applying successive identical square wave current pulses of about 100 nA under current clamp conditions and recording the changes of membrane potential. A membrane hyperpolarized by a given current under resting conditions became partly depolarized by nerve stimulation, by dopamine and by added potassium, while the opposite effect (hyperpolarization) was produced if the clamped current was zero. These results clearly link the effect of the nerve impulse with release of dopamine or a related transmitter and with increased permeability to potassium. The quantitative interpretation rested upon assumptions as to the applicability of the Nernst (Nernst-Planck) equation and some inconclusive discussion ensued. It was also noted that the membrane potential changes were damped, presumably because of a capacitative element omitted from the original model circuit.

Photosynthesis

Quite properly, in a symposium of this sort, the four papers on photosynthesis focused upon a minute, but essential, step in the utilization

of radiant energy in green plants and plant cells. Increasingly, in recent years, research in photosynthesis has been enabled by advances in optical techniques to probe the unique early stages of photon transformation. Consequently this type of work has taken precedence over the immediately less exciting study of the subsequent biosynthetic processes, the principles of which have probably been unearthed already by conventional biochemical study of photosynthetic and non-photosynthetic systems.

A brief introduction will serve to pin-point the domain of these recent investigations. Photosynthesis used to be represented conventionally by the equation:

$$H_2O + CO_2 \xrightarrow{nh\nu} CH_2O + O_2 \qquad (1)$$

with the stipulation that the necessary energy is provided by light (here represented by $h\nu$) absorbed by chlorophyll and other chromophores in the plant cell. The equation is misleading in the sense that carbon dioxide is not actually involved in the early photophysical and photochemical changes, so that the overall reaction must be broken down into photic and thermal components. The photic reactions can be represented by the following three equations:

$$H_2O \xrightarrow{nh\nu} 2H^+ + 2e^- + \tfrac{1}{2}O_2 \qquad (2)$$

$$NADPH^+ + 2H^+ + 2e^- \xrightarrow{nh\nu} NADPH + H^+ \qquad (3)$$

$$ADP + P_i \xrightarrow{nh\nu} ATP \qquad (4)$$

where the symbol $nh\nu$ is used to represent photon participation without any implication concerning mechanism or quantum efficiency; H^+ stands for proton, e^- for electron; $NADP^+$ is oxidized triphosphopyridine nucleotide, NADPH is the reduced form; ADP is adenosine diphosphate, ATP is the triphosphate; P_i is inorganic phosphate. The thermal reactions which follow result in carbohydrate formation from carbon dioxide, following a reversed glycolytic pathway energized by ATP and NADPH and catalyzed by organic substances which are regenerated cyclically along the pentose pathway so as to permit the overall process to continue with a net gain in carbohydrate.

The site of these reactions is thought to be the thylakoid, a flat lozenge-shaped sac containing chlorophyll and other pigments and catalysts enveloped by a membrane and stacked to form "grana" within the specific plant organelle known as the chloroplast. Within each thylakoid, incident light encounters some 10^5 so-called "antenna" molecules of cholorphylls a and b and carotenoids. If absorbed, the energy of excitation is transferred by some sort of migration of electronic

states and holes (sites vacated by electrons which are raised to higher energy levels) from molecule to molecule, and delivered to one of about 200 electron transport loci. At these loci reactions (2) to (4) occur, with evolution of oxygen and formation of NADPH, ATP and free protons. These reactions are thought to occur by electron transfer through two reaction centers in series. Reaction (2) takes place as a result of singlet state excitation of chlorophyll a_{II}, a form of chlorophyll which probably has an absorption maximum at 690 nm. The excited chlorophyll acts, in effect, as a catalyst removing an electron from water and passing it uphill to an unknown acceptor:

$$(\tfrac{1}{2}H_2O).Chl_{II}.A \xrightarrow{h\nu} (\tfrac{1}{2}H_2O).Chl_{II}^{*}.A$$

$$\longrightarrow (\tfrac{1}{2}H_2O).Chl_{II}^{+}.A^{-} \longrightarrow (H^{+} + \tfrac{1}{2}O).Chl_{II}A^{-} \qquad (5)$$

Acceptor A^- then initiates a series of reactions involving plastoquinone as electron donor and ferredoxin as acceptor, as well as cytochromes and other redox intermediates delivering the electron to a second reaction center occupied by chlorophyll a_I (absorption maximum 700 nm). The chlorophyll a_I also receives energy from the antenna system and upon excitation pumps the electron further uphill by a process similar to that in equation (5) but using a different donor-acceptor pair. The ultimate acceptor is $NADP^+$, and the product is NADPH.

The events just described utilize only part of the absorbed energy for production of NADPH. An attractive possibility that has been well thought of, inspired by Mitchell's proton translocation hypothesis, sees the electron transport and the concomitant proton production as proceeding vectorially between the inner and outer interfaces of the thylakoid membrane. The resultant proton efflux is thought by the proponents of the idea to be coupled to ATP production according to equation (4), with ATPase as catalyst.

The paper by H.T. Witt (Max Volmer Inst., Berlin, FRG) followed through (at excessive length) some demonstrated consequences of the postulated light-induced delocalized charge distribution across the photosynthetic membrane. The membrane potential generated in this manner would establish a gradient of the order of 10^5 V/cm, sufficient to produce a measurable effect upon the absorption spectra of the photosynthetic pigments by the electrochromic or Stark effect. (A point not mentioned is that this is of the same order of magnitude as the potential across many resting biological membranes, so that even in the dark it must be assumed that the pigments are modified by local electrical conditions.) Witt and colleagues were able to measure the very complicated difference spectra caused by flash illumination of spinach chloroplasts and photosynthetic bacteria; they covered the wavelength range from the Soret band at about 400 nm to 700 nm and

showed also that the absorption change occurs on roughly the same time scale as the reaction sequence in photosystems I and II (rise time less than 20 ns, dark decay about 20 ms). An *in vitro* Stark effect generated by applying an electric field across multilayers of chlorophylls a and b and carotenoids gave rise to a difference spectrum which agreed well with that produced by illumination *in vivo*. A direct demonstration of a light-induced transmembrane potential, first done by Fowler and Kok, was ingeniously performed by illuminating a suspension containing 10^9 thylakoids from one direction and recording the potential difference between electrodes immersed at different depths in the suspension. Absorption of the incident light made the system unsymmetrical so that a net potential difference between light and dark regions was created. When the effect was magnified by adding gramicidin to increase the proton flux and when interference from external ion fluxes was reduced by suspending the thylakoids in a viscous sugar solution, the measured potential change as a function of the number of stimulating flashes agreed quite well with changes in the Stark effect determined spectrophotometrically.

It was mentioned earlier that the protons "left over" from reactions (2) and (3) and vectorially disposed in the photosynthetic membrane were thought to provide the energy necessary for phosphorylation of ADP. Witt, in considering this point, used the light-induced absorption change at 515 nm as a transmembrane voltmeter. The polypeptide gramicidin was added in order to cause leakage of protons, bypassing ATPase and the rest of the system by which proton efflux is coupled to ATP production. The field-indicating absorption changes decay much more rapidly in the presence of gramicidin, and the involvement of the normal field in ATP production is - according to Witt -- proved by his finding that the curve for log τ (τ being decay time) as a function of gramicidin concentration is similar to the curve showing decrease of ATP production. The correlation is indeed striking, covering a range of 200 to 50 ms for τ and 0.2 to about 0.02 molecules of ATP per flash and electron chain.

The question that was inevitably asked is: If the transmembrane potential *per se* causes ATP production, is photon absorption needed at all? If not, then the whole photosynthetic meachinery - as far as ATP is concerned - could be replaced by an applied transmembrane potential. This seems to be the case, for voltage pulses (200 V, 30 ms, calculated to give a transmembrane potential of 200 mV) were found to generate ATP proportional to the number of pulses and at a rate comparable to that caused by light. A first thought is that the field effect could be due to an induced conformational change in membrane ATPase. In reply to questions, Witt said that the local pH gradient attributable to electrophoresis is too small, by a factor of 10^4, to account for the observed results. He noted also that

symmetrical ATP formation on one side and ADP on the other, suggested by Joliot, does not occur because of the large excess of ADP concentration in the system.

The discussion was disappointingly cursory, for the reason already implied. The latest results on ATP formation in an electric field clearly deserve close scrutiny for they carry important implications with regard to the Mitchell proton gradient hypothesis. Further, it will be necessary to show whether rate of absorption of electrical energy agrees quantitatively with that needed for the observed rate of ATP formation.

Electron transport performed by photosystems II and I has been thought, in line with Mitchell's hypothesis, to follow a zig-zag course, the physical basis of which could lie in the location of the reaction centers, Chl_{II} and Chl_I within the thylakoid membrane. P. Joliot (Inst. de Biologie Physicochimique, Paris) selected the electrochromic decrease of absorption at 480 nm and the increase at 515-520 nm as a measure of intra-membrane charge separation. The changes were found to be biphasic. The initial change was too rapid to resolve, but the slow second phase followed the same kinetics at both wavelengths. By examining these changes and their partial suppression by hydroxylamine, which inactivates the secondary electron donors of photosystem II, Joliot arrived - by arguments that I failed to follow - at the conclusions that the Chl_{II} is outside the membrane or very near to the outer surface, and that Chl_I is well inside the membrane, while the secondary electron donors and acceptors of photosystem I are situated respectively at the outer and inner surfaces. This much could be gathered from the abstract. The model differs, I think, from that of Witt, who placed Chl_{II} and Chl_I at positions in the middle of the membrane so as to cause the electron transfer to follow a zig-zag course of "internal reflections" between the two surfaces.

R. Kraayenhof (U. Amsterdam, The Netherlands) used a series of electrostatically and covalently bound fluorescent probes to demonstrate light-induced formation of negative charges on the thylakoid surface and a change of conformation in chloroplast ATPase.

The static electric field created in a dielectric chloroplast membrane during the primary photosynthetic separation of charges has been examined theoretically by G. Paillotin, M. Michel-Villaz and H. Conjeaud (Centre d'Etudes Nucléaires de Saclay, Gif-sur-Yvette, France), taking into account the effect of the distribution of charges induced on the surface of the membrane, which reduces the energy required and stabilizes the final configuration. The results were presented in a series of space contour diagrams showing the field

strength produced by charge pairs as a function of distance of separation of charges and distance from the center of the membrane. The contours are so steep that interaction between pairs can be neglected. These results were considered in terms of the Stark effect. The discussion which followed showed that the treatment offered did not account for the vectorial nature of the actual process nor for the absence of a back reaction. One had the impression that this work will only become directly applicable to some of the hypotheses proposed on experimental grounds after it has been extended to a more elaborate model.

Visual Transduction and Energy Conversion By Rhodopsins

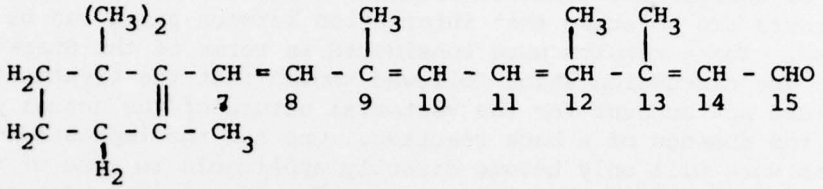
Beta carotene has already been mentioned as a common constituent of photosynthetic tissues. Its derivative, vitamin A, is to be found at two extremes of biological organization, namely in certain bacteria and in vertebrates. In the form of remarkably similar complexes vitamin A subserves in these organisms two different functions. In the bacteria it acts as a converter of light energy by creation of a proton pump for ATP synthesis. In the vertebrate eye it is a visual transducer mediating sodium ion transport across a membrane and so generating nerve impulses from a photic signal.

A. Lewis (Cornell U., Ithaca, N.Y.) presented recent studies of both systems, pointing out differences which, he thought, are related to their divergent functions. As in the papers on photosynthesis, that of Lewis dealt almost entirely with the new understanding of the primary photophysical and photochemical processes that has resulted from recent technical advances, saying little about the subsequent chemical and electrochemical coupling that enables the needed biological task to be performed.

First let me provide a little background information which some in the audience might have been grateful for. In some bacteria and in the vertebrate eye the chromophore is retinal, the colorless aldehyde of the alcohol vitamin A (retinol), which becomes blue or purple when modified by condensation with the ε-amino group of a lysine residue in a membrane glycoprotein, resulting in formation of a Schiff base:

$$R.CHO + H_2N.CH_2R' \longrightarrow \underset{(X)}{R.CH{=}N.CH_2R'} + H_2O \quad \ldots(1)$$

Here R.CHO stand for retinal, a conjugated polyene:



which is usually in the form of the 11-*cis* isomer. R' stands for the membrane protein with lysine side chain. When the Schiff base X is the photo-receptive pigment in the retinal rods, it is called rhodopsin and the protein R' is opsin - not necessarily of constant composition. The pigment in the retinal cones is called iodopsin and R' is photopsin. Compound X isolated from the purple halophilic bacterium *Halobacterium halobium* is called bacteriorhodopsin.

The primary photo-event is absorption of light by the chromophore. This is succeeded by a series of thermal events with ultimate regeneration of retinal and its reduction to retinol. The main absorption maximum of rhodopsin is at about 500 nm, that of bacteriorhodopsin at 570 nm. The first major products of absorption in each case are the corresponding batho-compounds, bathorhodopsin and bathobacteriorhodopsin, which show a red shift with maximum absorption at 543 nm and 635 nm respectively. The subsequent thermal transitions result in absorption shifts toward the blue.

As Lewis implied, a milestone in the study of visual transduction was the conclusion of Wald and associates around 1957 that the key reaction produced by light is the *cis-trans* isomerization of the rhodopsin chromophore. There the matter seems to have rested, for technical reasons, until the advent in the last few years of resonance Raman spectroscopy and picosecond emission and absorption spectrophotometry. Before trying to summarize the conclusions Lewis drew from his studies (with Stoeckenius and others) using these methods, led me add a little to his description of the methods themselves.

Ordinary Raman scattering measurements would be of little use in the present context. Their lack of selectivity would lead to insuperable difficulty in assigning vibrational levels. In any case, the signals might be obliterated by fluorescence. Raman lines are greatly enhanced when the incident radiation frequency is near that of an absorption band. With a tunable dye laser it becomes possible to scan the excitation profile of different vibrational modes and even to measure excitation times. Since the vibrational spectrum of a chromophore is highly sensitive to structural

changes and to environmental influences, resonance Raman spectroscopy should be a valuable tool for study of the rhodopsin reaction sequence.

Picosecond emission and absorption spectrophotometry also owes its feasibility to laser technology. In the arrangement used by Lewis a beam splitter sends part of the laser beam through a chopper to the sample and part to a motor-driven 90° reflecting prism. The fluorescence and the reflected laser pulse arrive simultaneously at the beam splitter only, of course, when simple conditions are satisfied in terms of the laser pulse duration (10 ps), the relative transit times from beam splitter to sample and from beam splitter to prism, the pulse pattern of the chopper, and the fluorescence kinetics of the sample. Background light from the main laser beam is eliminated by passing the mixed light through a lithium iodate crystal in which frequencies are combined additively to give a resultant remote enough from that of the source to be easily measured in intensity and phase after passing through a monochromator and phase-sensitive detector. For example, if excitation is at 590 nm and emission at 772 nm, the combined wavelength will be 334.4 nm. Computer processing of the signal as a function of delay time, given by the position of the reflecting prism, leads to the desired emission decay curve.

The results on resonance Raman spectroscopy reported by Lewis appear to identify the Schiff base linkage as the locus of the functional mechanism in all rhodopsins, drawing attention away from the isomerization to which so much importance had been attached. The *cis* and *trans* isomers of retinal can be distinguished in the resonance Raman spectra of their crystals. Applying this information to illuminated rhodopsins, it is shown that the data "are inconsistent with a formal *cis* - *trans* isomerization." The differences between rhodopsin and bacteriorhodopsin in this regard seem not to have been securely established, for the literature contains conflicting statements, but Lewis insisted that bacteriorhodopsin has managed to avoid conformational change because of the need for reversibility as an energy converter, while for visual transduction irreversibility is needed, and this is provided by the *cis* - *trans* reaction. The more significant results to come out of resonance Raman studies are that the Schiff base linkage exists in the protonated form

$$\text{—N}_{\text{H}^+}\text{=}$$

rather than that shown in equation (1) and that the proton is released by some mechanism linked to a primary electron delocalization process in the conjugated chain and subsequent relocalization in the thermal intermediates arising from bathorhodopsin. Assuming that the pigment releases a proton on one side of the membrane and later takes up a proton on the other side, a transmembrane proton

gradient can be established and the conditions are satisfied for a proton pump consistent with Mitchell's well-known hypothesis. This deprotonation of the Schiff base has been found in all rhodopsins studied and can therefore be considered as the essential first step toward ATP synthesis or initiation of transmembrane sodium ion flow.

Lewis then described two experiments on the picosecond kinetics of bacteriorhodopsin. In the first, it was possible to detect a transient increase in absorption of bacteriorhodopsin at 615 nm about 0.5 ps after illumination with a sub-picosecond laser pulse, presumably at 570 nm. This seems to show that bathobacteriorhodopsin is formed too quickly to allow any change of conformation. The second experiment showed that bacteriorhodopsin illuminated at 580 or 590 nm emits a red fluorescence with an emission lifetime of 14 $\pm$ 1 ps. These results taken together suggest that during the formation of bathorhodopsin there is some vibrational relaxation into a low energy state which by light emission reverts to bacteriorhodopsin. Although this added to the detailed picture, it is, I imagine, a side effect of little direct significance, the quantum efficiency being about 2.10^{-4}.

3. NON-VIABLE SYSTEMS

Scattered among the "biological" papers reviewed in Section 2 were a roughly equal number dealing with laboratory preparations that exhibit some of the properties of living cells and organisms. We are on familiar ground, and we shall expect to find much emphasis on the incidental properties of cells and a notable lack of success in reproducing what we consider to be uniquely biological characteristics. The idea is that one looks for inert systems which show some structural or organizational feature that we suppose to underlie biological activity. Then, having learned more about them as physical or physicochemical objects, one proceeds to incorporate catalysts, substrates and energy sources in the hope that if this is done with appropriate subtlety they will become capable of organic synthesis, excitation, contraction, or locomotion, to say nothing of self-replication.

The papers about this sort of thing fought shy of any commitment to such a *mode d'emploi* of their authors' favorite objects, but progress toward the building of genuine models having one or another of the elementary properties of cell components is discernible. Had the symposium attained its putative objective, there would have emerged a continuing dialogue between the real biologists and the others who play with models. This was not to be. What started as a series of papers ended as a series

of papers, clarified only by fragments of discussion among specialists.

Monolayers at Air-water Interface

It must suffice to mention that I. R. Miller (Weizmann Inst., Hehovot, Israel) described dynamic electrical changes attending modifications of spread lipid monolayers by electroactive compounds and by film-penetrating proteins and polypeptides. The electrical measurements, made with a dropping mercury electrode, gave values of specific capacitance and current-voltage curves, while estimates of permeability were made by oxygen- and ion- polarography. The experimental set-up is such that a good deal of interpretation is needed, particularly of "pseudocapacitance" peaks resulting from special electrode reactions with electroactive groups--including disulfide groups in the protein penetrants.

I was sorry to miss the paper by L. Ter-Minassian-Saraga (U.E.R. biomédicale dès Saints-Pères, Paris) because she and her pupils seem to have found properties of model membrane protein monolayers that have their counterpart in nerve and muscle, especially featuring the role of membrane potential in controlling sodium ion transport. Since the penetration of mobile ions is shown (according to the abstract) to depend specifically upon the amino acid composition of the protein, one wonders whether in such a relatively simple system as a monolayer some change analogous to "gating" will eventually be observed.

Monolayers on Metals

R. Bennes, E. Bou Karam and D. Schuhmann (CNRS, Montpellier, France) used monolayers of polar species--compounds of no particular biological significance--adsorbed from solution onto a mercury surface as a model for the hydrophilic outer layers of bimolecular lipid films. Parallel measurements on bilayers were reported by C. Gavach, also from Montpellier.

Multilayers

The paper by G. Stulen (U. Groningen, The Netherlands), who was not present, was to have described the effects of external electric fields upon a stack of about 1000 phospholipid bilayers between metal-coated coverslips. Changes in organization were monitored by electron spin resonance of added fatty acid and cholesterol spin labels. Various reversible effects attributable to partial disorientation take place, and the results would have been worth discussing in relation to field-induced changes in photosynthetic and other biological membranes.

C-3-77

Lipid Bilayers

The papers describing experiments on bilayers brought us closer to the properties of biological membranes: formation and nature of conducting pores; adsorption of charged hydrophobic molecules; mobility and transport in the plane of the membrane.

Much use has been made both *in vivo* and *in vitro* of the decapolypeptide gramicidin and the octadecapeptide alamethicin for channel generation. P. Läuger (U. Konstanz, FRG) considered the conductance of lipid bilayers exposed to gramicidin in terms of a head-to-head dimer, 30 Å long, forming a tunnel 3 Å in diameter, lined by the carbonyl groups of the peptide residues. Following some work of Haydon, he found that upon addition of low concentrations of gramicidin discrete transmembrane current fluctuations occurred. These could be attributed to the formation of single conducting units by single molecular events, and the unit conductance and pore lifetime could be determined. The kinetics supported the idea of a dimeric molecular unit, and it was shown also that the peptide can pass through the membrane. A negatively charged substituted molecule, O-pyromellityl gramicidin A, produced only a small transmembrane conductance when applied on one side of the bilayer and a much larger conductance when put on both sides. The negative charge apparently prevents passage through the membrane, and the high conductance evidently arises from the meeting of channels formed by penetration in both directions, the negative charges remaining at the two channel entrances. This observation precludes one suggested model which postulated dimerization in the form of a double helix. In presence of a mixture of the parent peptide and its negatively charged derivative the probability of channel formation on a picosecond scale shows three peaks, one each for the two compounds and a smaller one for hybrid channels.

The channels are blocked by thallium ions which cross the bilayer and by divalent cations such as Ca^{++} and Ba^{++} which do not. These ions behave as if they exercise a direct influence on single pores by becoming bound near the mouth. No mixed blocked and unblocked currents have been observed. The rate of blockage is about 10^8 to 10^9 s^{-1}, close to that expected for a diffusion-limited process.

Discussion brought out the statement that no effect of bilayer compression on pore formation had been demonstrated. It was mentioned also that once a membrane current has been established with a resolvable time constant, voltage reversal causes square wave current reversal. The interesting question was raised of rectification by putting different ions on opposite sides of the bilayer, but the experiment had not been tried.

A slightly different approach on the part of R. H. Tredgold (U. Lancaster, UK) led him to study the dielectric properties of dry

polypeptide crystals and to do experiments by Läuger's technique on the single channel conductivity of bilayers containing gramicidin analogues. The energy of dehydration of a univalent inorganic cation, calculated from the Born equation and the Pauling radius, is several eV , much more than 40 kT. This would be the energy needed to transfer the ion from an aqueous to a hydrocarbon environment. Thus, to permit ion permeation, the local dielectric constant must be considerably increased. Speculating that this might be accomplished, for example, by incorporation of a glycoprotein with the peptide chain embedded in the lipid tails and the carbohydrate in the axial region constituting the pore, Tredgold was led to the studies already mentioned.

A series of polypeptides in crystalline beta-pleated sheet form was prepared by a computer-programmed Merrifield synthesis. The dielectric dispersion was measured using films 2 μm thick deposited upon mercury from a solution in dichloracetic acid and maintained in an a atmosphere of dry nitrogen. The real dielectric constant ε' was between 20 and 30 and not steeply frequency-dependent between 0.1 and 10 MHz and the imaginary component ε'' was very low. The calculated dipole moment was about 3.7 Debyes per peptide bond. Thus pores lined with such compounds would seem to have the required characteristics for ion penetration.

The measurements on bilayers containing gramicidin analogues, likewise prepared by the Merrifield method, supported the hypothesis of oriented polypeptide pores in which the peptide linkages can be rotated into potential energy configurations favorable to ion dehydration.

In discussion Tredgold expressed himself sceptical about the value of dielectric measurements on protein solutions in the present context, since these contribute information only about the water. It is presumed that ions permeating a bilayer enter a polar environment free of water molecules. Within the pore they will generate a radial dipole moment across the polypeptide which did not pre-exist (contrary to a supposition made by Läuger) and which presumably affects the kinetics of their passage.

In his paper on hydrophobic adsorption of charged molecules S. McLaughlin (State U. New York, Stony Brook, N.Y.) used the Stern equation as reference point for experimental data of several kinds. Adsorption of such molecules, even if thermodynamically favorable from the standpoint of hydrophobic-hydrophobic contacts created by elimination of water, modifies the electrostatic potential at the surface and so depletes the solution in the immediate vicinity of the surface of dissolved adsorbate molecules. The Stern equation applicable to this situation results from three relationships: the Gouy equation for the diffuse double layer, the Boltzmann equation for ion distribution

in an electric field, and an expression similar to the Langmuir isotherm, or the limiting form of the Balmer equation, for adsorption at fixed sites. McLaughlin gave these equations in the following form, without going into Stern's derivation or his correction for finite ionic size:

Gouy: $\sinh(F\psi/2RT) = 136\sigma c^{-\frac{1}{2}}$

Boltzmann: $[A^-]_o = [H^+] \exp(F\psi/RT)$

Langmuir: $$\sigma = \frac{\sigma_{max}\,[A^-]_o}{K + [A^-]_o}$$

where F is Faraday constant, ψ is surface potential, σ is surface charge density, c is concentration, $[A^-]_o$ is concentration of adsorbate ions close to the surface, $[H^+]$ is counterion concentration in the ambient solution remote from the interface, σ_{max} is the surface charge density when all adsorption sites are occupied, and K is a constant. The resulting Stern equation can be tested by measuring both surface charge density and surface potential as a function of bulk phase concentration of adsorbate.

McLaughlin cited values of the surface charge density derived from the published data of C. Huang and J. P. Charlton who had shown the adsorption of 2,6-toluidinylnaphthalenesulfonate (TNS) by phosphatidylcholine vesicles, represented by a Scatchard plot, to be consistent with the Stern equation. In his own experiments he studied adsorption of the same substance by measuring the electrophoretic mobility of Bangham phosphatidylcholine multilayer vesicles suspended in TNS solutions of different concentrations, reporting the results graphically in terms of zeta potential and log concentration. Finally, the surface potential of planar black lipid membranes was measured by use of tetraphenylborane as an ionic probe which can pass through the membrane under an applied potential gradient. The current generated is a measure of the number of ions adsorbed. It was not clear (to me) how the surface potential was derived from this information, the matter being complicated by unexplained saturation effects and by the large potential change within the membrane when the ion enters a region of low dielectric constant. Nevertheless McLaughlin considered that the evidence from the three types of measurement is cumulatively favorable to the Stern equation in its simplified form, regarding ions as point charges.

Phospholipid Vesicles

C. Sauterey, giving the paper by C. Taupin, M. Dvolaitzky and himself (Collège de France, Paris), introduced their experiments on pore formation in phospholipid vesicles by referring to differences in the rates of "flip-flop" exchange between inner and outer lipid components associated with differences in composition. Vesicles of dipalmitoyl lecithin (DPL) are leakier, and their boundary bilayers undergo more rapid non-linear flip-flop than vesicles of egg lecithin (EL). Attributing the flip-flop process to lateral diffusion around pores in the bilayer, Taupin and colleagues attempted to find a correlation between these properties and leakage of solutes through pores generated by osmotic stress. Osmotic stretching was brought about by a difference in sodium chloride concentration between vesicle contents and suspending solution, and leakage was measured by use of a hydrophilic spin-labeled choline derivative. The results were considered in relation to a simple theory of pore formation involving a quadratic activation energy dependent upon interfacial energies and pore diameters, and showing the familiar tendency of pores to collapse when smaller than a critical value and to expand when greater. Differences between EL and DPL vesicles in flip-flop exchange and readiness to fuse correlate well with differences in rate of nucleation for pore formation as estimated from these measurements of leakage rates as functions of sodium chloride concentration gradient and temperature.

Lateral Transport in Membranes

It is no longer appropriate to regard biological membranes as fixed structures nor even as possessing a static framework within which clearly defined receptors or sites of biological reactivity are embedded. The postulted lipid bilayer core itself may well be subject to phase transitions accompanied by rheological change, and the possibility of relative motion in the plane of the bilayer as well as local change of molecular configuration normal to the bilayer must be entertained. Similarly the protein films originally thought to coat both surfaces of the bilayer might be able to diffuse or to flow with respect to the core. These questions have become more pressing as membrane models, under pressure of experimental evidence from many directions, have become increasingly elaborate. It has become necessary to understand how some membranes, in order to do their job, must have zones of reactivity which are constrained and immobile while others must permit diffusion on a molecular scale, mass flow, or vectorial relative motion of components in a heterogeneous matrix.

Once again the laser has provided powerful new means of investigating these questions. An impressive review was given by W. W. Webb (Cornell U., Ithaca, N.Y.) of work in his laboratory on the use of f fluorescent probes to measure lateral motion on the surface of membranes and films. The two methods used permit mass flow to be distinguished from diffusion, and diffusion coefficients over the range 10^{-14} to 10^{-5} cm^2s^{-1} can be measured. The two methods employ essentially the same experimental set-up, which provides laser illumination of a spot about 1 to 10 μm^2 on the surface of a specimen labeled with a fluorescent probe and permits measurement of the intensity and polarization of the excited fluorescence as a function of time.

For rapid changes the system need not be perturbed other than by illumination sufficient to excite measurable fluorescence in an area of about 1 μm^2. Stochastic fluctuations of fluorescence intensity are measured. These fluctuations, taken over intervals t and $t + \tau$, permit computation of experimental estimates of an autocorrelation function from which the origins of the fluctuations can be discriminated and values of the characteristic times for diffusion and/or uniform flow estimated. Further, when $\tau = 0$, the extrapolated function is simply and usefully related to the number average, $\overline{N}$, of independent fluorescent particles in the field. For a monodisperse system,

$$g(\tau=0) = \left\langle (\delta I)^2 \right\rangle / \left\langle I \right\rangle^2 = 1/\overline{N}$$

where the quotient is the normalized variance of intensity fluctuations δI. This has been put to use in determination of molecular weights and degrees of aggregation of intrinsic membrane components and probes.

When observation must be continued for longer periods (up to 10^3 s, compared with less than 1 s for autocorrelation fluorimetry), a somewhat larger sample spot--say 10 μm^2-- is irreversibly bleached, and the restoration of fluorescence by centripetal migration of unbleached fluorochrome is monitored. The fractional recovery as a function of time is compared to theoretical curves for diffusion and/or uniform flow. Not surprisingly, the theoretical curves for the two methods are similar in form.

Webb presented a great deal of information obtained by these methods, using as fluorochrome a carbocyanine dye which is not endocytosed. The objects of study ranged from "dry" bilayers above and below the transition temperature to the surfaces of cells treated with concanavalin A and its antibody. There was also some mention of "synthetic" cells made by incorporating the so-called "universal"

membrane protein in black lipid membranes, and of two-dimensional antigen-antibody reactions in such a system. Webb exhibited a slide containing a lengthy and, no doubt, informative list of things that would be worth doing now that these new methods are available. Unfortunately the list was visible only to people close to the screen or armed with opera glasses and in any case barely assimilable in the few seconds of permitted viewing time.

The discussion reflected some misgivings about the nature of the processes being studied, particularly as these may be affected by local heating and by reactions with the deeper layers of the cell membrane or the cytoplasmic substrate. Webb fielded the questions with skill, demonstrating that there were few doubts that had not already been raised within his own circle of investigators. Indeed, his critical and constructive contributions throughout the symposium were notable, even if marred now and then by a trace of condescension toward his less fortunately situated European colleagues.

Electron transport

The mechanisms of electronic conduction in condensed phases were reviewed by M. Schott (U. Paris VII) immediately before the group of papers on photosynthesis. The last paper of the week was by B. Karvaly (Hungarian Academy of Sciences, Szeged, Hungary) on semiconductor properties of lipids and bimolecular lipid membranes. Measurements of the activation energy for conduction agree with dielectric data in suggesting a hopping mechanism involving excitons. Another paper in the final session, by R. Buvet (U. Paris XIII), Créteil, France) dwelt on the potentials of electron exchange substances such as solid polyalanine in different redox states. In membranes constituted of such substances the existence of a membrane potential will introduce a Donnan effect. With abolition of the potential by change of external pH or of redox state, the Donnan effect will disappear and the permeability will increase. One would have to listen at greater leisure to decide whether there was anything new in all this.

At risk of being accused of parochial prejudice I must say that these papers were greatly outclassed by one given by J. J. Hopfield (Princeton U., N.J., and Bell Telephone Laboratories, N.Y.). After reviewing most lucidly the theory of electron transport and comparing Förster excitation transfer with the tunneling mechanism, Hopfield considered the primary reactions in bacterial photosynthesis as described by DeVault and Chance: i.e., the transfer of electrons from cytochrome c through photoexcited bacteriochlorophyll to a

primary acceptor. At low temperatures the rate becomes independent of temperature and the process is therefore supposed to proceed by a tunneling mechanism. The theory of this leads Hopfield to predict that there will be a weak infrared charge-transfer absorption band of very low oscillator strength, with a maximum molecular extinction coefficient of 0.3, at about 1.3 μm. The difficulty of measuring such a weak absorption can be overcome, he hopes, by using the model system consisting of cytochrome c and ferricyanide. This mixture is to be illuminated continuously at the wavelength of the Soret band and intermittently by infrared radiation. If the infrared produces a change in the redox state of the cytochrome, the intense Soret band will be shifted and an ac signal will appear. The results of this ingenious experiment were not available.

Theory of Membrane Properties

The non-equilibrium thermodynamics school of Prigogine was represented by R. Lefever (U. Libre de Bruxelles, Belgium) who posed questions regarding non-equilibrium boundary conditions in membranes. He considered various types of reaction sequence and their consequences, showing that a third order component is needed in order to produce a multiplicity of stable steady states of different probabilities. Some of these combinations of steady states with intervening metastable zones may lead to the possibility of wave functions and, applied to nerve, might account for observed rates of change of permeability during excitation. Discussion brought out the admission that these approaches do not always provide unequivocal criteria for distinguishing equilibrium from steady state.

Descending from thermodynamic eminences to the molecular level, M. J. Sparnaay (Philips Research Lab., Eindhoven, The Netherlands) after announcing a forthcoming book on surfaces, attempted to calculate the stress-strain properties of straight chain aliphatic monolayers with single polar end groups. This was done by summing the chain interaction energies as a function of mutual orientation in a plane. The basic datum is the van der Waals interaction energy for methane, cited as 4.4×10^{-58} erg cm^6. The results for two aligned rods of length l and distance r apart give proportionality with $l^2 r^{-6}$ for large separations and $l r^{-5}$ for close approach. From this, values of Young's modulus can be calculated.

Sparnaay then went on to discuss the mechanical properties of lipid bilayers. He introduced the idea of using an applied potential difference to produce an effective change of interfacial tension and so to derive a stress-strain function.

From the abstracts I gather that A.P.R. Theuvenet and G.W.F.H. Borst Pauwels (U. Nijmegen, The Netherlands) cautioned against the serious errors that may arise from failure to take into account the effect of ions on surface potential when one is trying to interpret ion-induced changes of ion translocation kinetics. An example quoted is the apparent competitive inhibition of rubidium ion uptake in presence of polyvalent cations, which they attribute to polycation binding to negative fixed surface charges rather than to competition for translocation sites.

Also from the abstracts I gather that Yu.A. Chizmadzhev (USSR Academy of Science, Moscow)--who was not present--intended to proceed from a description of ion transport by mobile carriers and through channels to calculation of unidirectional ion fluxes by single-file motion in biological membranes. Thence he would have gone on to derive a scheme which would reproduce experimental data on the equilibrium and kinetic characteristics of membranes, including, for example, gating currents and electric fluctuation spectra. The additional assumptions needed for the model include sets of interacting dipoles corresponding to each channel conformation.

Electrical Noise in Membranes

There were to have been three papers on electrical noise in membranes, but V. A. Tyagay (Ukrainian Acad. Sci., Kiev, USSR) was represented neither in person nor as an abstraction. C.P. Bean, giving a paper by Bean and D.C. Golibersuch (General Electric Research and Development Center, Schenectady, N.Y.) introduced the question in an attractively informal way by exhibiting the first page of Jean Perrin's paper "Mouvement Brownien et Réalité Moléculaire," published in 1909. He then proceeded to suggest on statistical grounds that life may be prolonged by the study of fluctuations. The evidence for this startling idea is that the average life span of six outstanding investigators born between 1773 and 1889 is 82.3 ± 6.8 years. A seventh, M. Smoluchowski, lived to be only 45, a datum that had to be discarded, presumably on the ground of its unrealistic remoteness from the decisive average--unless, indeed, it is meant as a reflection on the quality of Smoluchowski's work, which few would have the temerity to cast in the absence of this supposedly objective evidence. Bean further endeared himself to a rather tired audience by providing xerox copies of his slides as an *aide-mémoire* which served too as a memento of the occasion.

Electrical noise was first illustrated in Bean's talk by reference to the principles of the resistance pulse (Coulter) counter in which the resistance across an aqueous pore is increased during passage of a non-conducting particle. The electrical signal will consist

of discrete pulses, for example, at a sufficiently low partial volume fraction, becoming irregular or "noisy" when the concentration is increased. Then the distinction was drawn between equilbrium thermal noise, in which the mean square voltage fluctuation is proportional to resistance, and excess noise which occurs only when there is a voltage difference and which carries information about dissipative processes. The linear dependence of thermal noise on resistance was represented by a single straight line in a plot of $(\overline{V^2})$ (range 1 to 5 x $10^{-12} V^2$) against R (0.1 to 0.5 x 10^6 ohm) for six different resistors: carbon filament, advance wire, and four electrolyte solutions.

The excess noise factor $\alpha(f)$ is calculated from the Fourier transform of the noise record. Shot noise, caused by electrons, is identified by proportionality of $\alpha(f)$ to f^{-2}, and flicker noise, of unknown origin, to f^{-1}. An equivalent approach is to use the autocorrelation function $A(\Delta t)$, equal to $\overline{V(t).V(t+\Delta t)}$, where V stands for signals at time t and $(t + \Delta t)$ and Δt is delay time. The application of this was illustrated with data on random square pulses produced by passage of polystyrene latex spheres through etched pores in mica or plastic. $[A(\Delta t)]^2$ decreases linearly with delay time, and passage time and linear velocity can be calculated. A practical limit of 80 Å diameter is set for study of particle noise because of the intrusion of ion fluctuation effects.

Ion-generated noise was studied by determining $[A(\Delta t)]^2$ as a function of the square of the applied voltage for a solution of HCl and for the equivalent metal film resistor. The plot is linear in both cases, but with roughly 8-fold greater slope in the case of the 1-1 electrolyte. The effect is as predicted for non-interacting identical particles-a puzzling result, since some inter-ion correlation would be expected.

A. A. Verveen (U. Leiden, The Netherlands) warned us in his abstract that a twenty-minute limit would make it difficult to do justice to the subject; the more complicated matters would have to be omitted. The result was quite complicated enough for this listener: too much so, I felt, for an inter-disciplinary occasion, no matter how childishly simple for those who are occupationally addicted to noise.

Verveen covered some of the same early ground as Bean though in a totally different style. He pointed out the equivalence of the autocorrelation function (loss-gain equation) and the spectral density function. He mentioned some of the types of behavior encountered in biological membranes and illustrated these by graphs of the spectral density function log $S(f)$ against log f: White

noise, in which $S(f)$ is constant; white noise at low frequency, succeeded by a Lorentzian or f^{-2} function at high frequency; f^{-1} noise and sometimes $f^{-1.5}$. There is no acceptable explanation for f^{-1} noise; it is often found and may sometimes be associated with diffusion in unstirred boundary layers. An empirical equation by Hooge expresses $S(f)$ as proportional to $\alpha N f^{-1}$ where N is the number of free charge carriers and α is a dimensionless "constant", the value of which, discouragingly, varies over four orders of magnitude. The $f^{-1.5}$ behavior is also unexplained.

In nerve, it is necessary to disentangle noise associated with several different processes: K^+ current, Na^+ current, leakage current, shot noise, excitation bursts, and perhaps chemical reaction components such as charge uptake by enzymes, which would generate very low frequency noise. There is evidence from the Hodgkin-Huxley work that K^+ current is controlled by four particles, Na^+ by three. There is also evidence for fluctuating channels and flipping transitions associated with gating. The case of single-file diffusion noise in membranes with one or two barriers has been exactly analysed by Läuger who predicts a sigmoid dependence of log $S(f)$ against log f, starting with white noise, decreasing with slope $f^{-\alpha}$ and levelling at high frequency. This behavior has not yet been seen experimentally.

4. CONCLUSION

In this report I have imposed a certain crude organization upon material which was presented during a far less-structured program. This I have done by segregation into living and dead systems: an artificial but still fairly respectable distinction. The easy way out perhaps. I had to recognize that an ideal confrontation between one and the other at all points of possible contact would prove to be as far beyond my capacity to meet any sort of realistic deadline as it was evidently beyond that of the organizers, who compromised by arranging certain rather unconvincing juxtapositions without taking the opportunity thus generated for real interdisciplinary interchange. One must not complain. This is not the first symposium, and it will not be the last, to fall short of what must have been impeccable intentions in the minds of the organizers. There were some lively discussions, and much evidence of the enormous amount of preparation that must have been needed in order to assemble such a gathering. The Société de Chimie Physique is to be congratulated upon its adventurousness.